

Endothelial Nitric Oxide Synthase (Tyr-657), phospho-specific [conserved site]

Cat. # NP4031

Host Rabbit Polyclonal

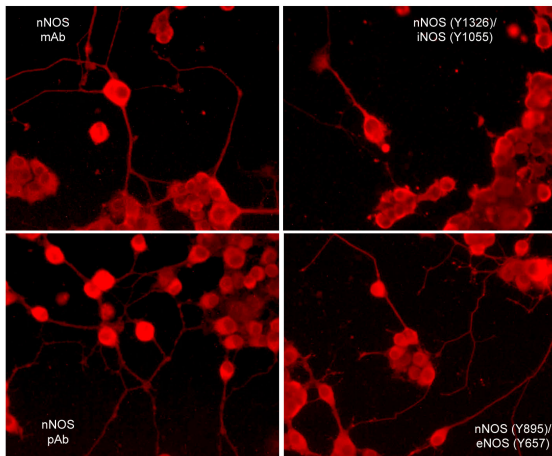
Size 100 µl

Background:

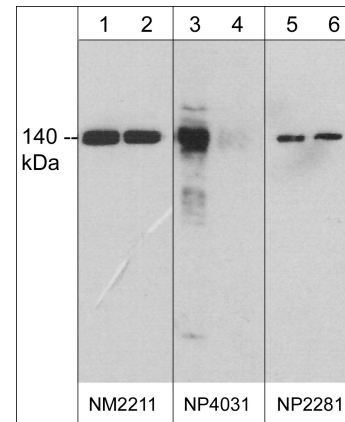
Nitric oxide (NO) has a broad range of biological activities and is implicated in signaling pathways in phylogenetically diverse species. Nitric oxide synthases (NOS), the enzymes responsible for synthesis of NO, are homodimers whose monomers are themselves two fused enzymes: a cytochrome reductase and a cytochrome that requires three cosubstrates (L-arginine, NADPH, and O₂) and five cofactors or prosthetic groups (FAD, FMN, calmodulin, tetrahydrobiopterin, and heme). Several distinct NOS isoforms are produced from three distinct genes, inducible NOS (iNOS, NOS-II), neuronal NOS (bNOS, NOS-I), and endothelial NOS (eNOS, ecNOS, NOS-III). Regulation of eNOS activity occurs through phosphorylation at multiple sites. Phosphorylation of Ser-633 (mouse Ser-632) in the FMN binding domain increases eNOS activity and may be important for the maintenance of NO synthesis after initial activation by Ca(2+) flux and Ser-1177 phosphorylation. Tyr-657 is phosphorylated by PYK2 in response to fluid shear stress and this phosphorylation leads to attenuation of enzyme activity.

References

- Musicki, B. et al. (2005) Proc. Natl. Acad. Sci. 102(33):11870.
 Mount, P.F. et al. (2007) J Mol Cell. Cardiol. 42(2):271.
 Fisslthaler, B. et al. (2008) Circ Res. 102:1520.
 Loot, A. E. et al. (2009) J Exp Med. 206(13):2889.



Immunocytochemical labeling of nNOS phosphorylation in rat PC12 cells differentiated with NGF. The cells were probed with mouse monoclonal (mAb) nNOS (NM4011), and rabbit polyclonal (pAb) nNOS (C-terminal region), nNOS (Tyr-895)/eNOS (Tyr-657), and nNOS (Tyr-1326)/iNOS (Tyr-1055). The antibodies were detected using appropriate secondary antibody conjugated to DyLight 594.



Western blot analysis of human umbilical vein endothelial cells stimulated with pervanadate (1 mM) for 30 min. (lanes 1, 3, & 5) then the blot was treated with alkaline phosphatase (lanes 2, 4, & 6). The blots were probed with anti-eNOS monoclonal antibody (NM2211; lanes 1 & 2), anti-eNOS (Tyr-657) phospho-specific antibody (NP4031; lanes 3 & 4), or anti-eNOS polyclonal antibody (NP2281; lanes 5 & 6).

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Cat. # NP4031
Host Rabbit Polyclonal
Size 100 µl

Immunogen:

Phospho-eNOS (Tyr-657) synthetic peptide (coupled to carrier protein) corresponding to amino acids surrounding tyrosine 657 in human eNOS. This sequence is conserved in mouse (Tyr-656) and rat (Tyr-656) eNOS, and is identical to the conserved site in nNOS (Tyr-895). The site is also well conserved in iNOS (Tyr-631).

Buffer and Storage:

Rabbit polyclonal, affinity-purified antibody is supplied in 100µl phosphate-buffered saline, 50% glycerol, 1 mg/ml BSA, and 0.05% sodium azide. Store at -20°C. Do not aliquot. Stable for 1 year.

Applications:

WB 1:1000
ELISA 1:2000
ICC 1:400

End user should determine optimal dilution for their particular applications and experiments.
Western blot membranes were incubated with diluted antibody in 5% non-fat milk, PBS, 0.04% Tween20 for 1hour at room temperature.

Specificity:

The antibody detects a 140 kDa* band on SDS-PAGE immunoblots of human umbilical vein endothelial cells treated with pervanadate, and this reactivity is not observed after alkaline phosphatase treatment.

*All molecular weights (MW) are confirmed by comparison to Bio-Rad Rainbow Markers and to western blot mobilities of known proteins with similar MW.

Related Products:

NX4035 phospho-eNOS (Tyr-657) Peptide
NM2211 Endothelial Nitric Oxide Synthase (C-terminal region) Mouse
NM2321 Endothelial Nitric Oxide Synthase (Ser-632), phospho-specific
NP2281 Endothelial Nitric Oxide Synthase Rabbit Polyclonal
NM4011 Neuronal Nitric Oxide Synthase (C-terminal region) Mouse
NM3981 Inducible Nitric Oxide Synthase (C-terminal region) Mouse

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